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Review

The ubiquitin- and SUMO-dependent signaling response to DNA double-strand breaks

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ABSTRACT

DNA double-strand breaks (DSBs) represent the most destructive type of chromosomal lesion and trigger rapid chromatin restructuring accompanied by accumulation of proteins in the vicinity of the DSB. Non-proteolytic ubiquitylation of chromatin surrounding DSBs, mediated by the RNF8/RNF168 ubiquitin ligase cascade, has emerged as a key mechanism for restoration of genome integrity by licensing the DSB-modified chromatin to concentrate genome caretaker proteins such as 53BP1 and BRCA1 near the lesions. In parallel, SUMOylation of upstream DSB regulators is also required for execution of this ubiquitin-dependent chromatin response, but its molecular basis is currently unclear. Here, we discuss recent insights into how ubiquitin- and SUMO-dependent signaling processes cooperate to orchestrate protein interactions with sites of DNA damage to facilitate DSB repair.

Among the thousands of DNA damaging insults encountered daily by any mammalian cell [1], DNA double-strand breaks (DSBs) represent the most cytotoxic lesion which, if left unrepaired, can be life-threatening for organisms as they alter the content and organization of the genetic material [2–4]. The cellular response to DSBs impacts on diverse cellular processes, such as DNA repair, cell cycle progression, DNA replication, and transcription [4–6]. This is in part achieved through phosphorylation of numerous effector components by the major DNA damage-responsive kinases ATM, ATR, DNA-PK, Chk1, and Chk2 [4–6]. In general, the plethora of phosphorylations mediated by these kinases enforce the desired DNA damage response by promoting or inhibiting the activities and/or functions of target proteins. A second and equally important way of fine-tuning the DNA damage response is through an elaborate compartmentalization process, in which signaling molecules are concentrated at, or excluded from, specific areas of the nucleus [7–9]. Both of these strategies are employed in the DNA damage response to activate, control, and facilitate the actual repair of DSBs. Thus, the majority of proteins with a direct role in DSB repair are targets of ATM, ATR, and DNA-PK, and in many cases these modifications have been shown to impact on the activity and/or complex formation of these proteins [6]. In addition, a protective and DNA repair-stimulating micro-environment is formed around the DSB sites. These structures, commonly referred to as Ionizing Radiation Induced Foci (IRIF), concentrate multiple DNA repair factors in the vicinity of the strand breaks, promote checkpoint signaling, and constitute a barrier towards further DNA decay [7,9].

High-resolution visualization of nuclear sites of DNA damage has revealed that IRIF consist of two spatially distinct compartments that provide specific binding platforms for proteins [10]. The first and larger compartment is formed by various modifications of the chromatin distal to the broken DNA, the most prominent being ATM-mediated phosphorylation of H2AX on Ser139, an epigenetic mark commonly referred to as γ-H2AX [11,12]. Following this modification, an extensive range of genome maintenance proteins accumulate in these regions of γ–H2AX-containing chromatin, in large part by recognizing specific chromatin modifications that are associated with the presence of DSBs [9]. The second, and comparatively much smaller compartment, is formed by a limited resection of the broken DNA ends, a process that creates long stretches of single-stranded DNA (ssDNA) [13]. This ssDNA is an intermediate in the repair of DSBs by homologous recombination (HR), but it also forms the structural base of the second DSB-associated compartment, to which a number of protein complexes bind [10]. These include most of the known HR repair proteins and factors involved in ATR-mediated signaling. DNA end resection only occurs in cells in S and G2 phases of the cell cycle, where the presence of an intact sister chromatid favors HR-mediated

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DSB repair [13,14]. Accordingly, formation of the ssDNA-associated IRIF compartment is also restricted to the S and G2 phases. This is in contrast to the larger chromatin-associated compartment, which is formed throughout interphase [10].

The accumulation of proteins on chromatin surrounding DSBs depends on DNA damage-induced chromatin modifications that are in turn recognized by a set of chromatin-binding complexes. To a large extent, the proteins that associate with these modifications are part of larger protein complexes or act as molecular scaffolds at sites of DNA damage, to which other proteins can bind transiently. Although phosphorylation has long been known to play a major role in orchestrating protein interactions with sites of DNA damage, it has recently become clear that other types of post-translational modifications (PTMs), in particular ubiquitylation and SUMOylation, are also critically engaged in such responses [9,15,16] (Fig. 1). The maturation of the chromatin compartment by DSB-induced PTMs occurs in a sequential fashion, where the initial ATM-induced phosphorylation of γ-H2AX provides a direct binding platform for the MDC1 scaffold protein [17]. MDC1 undergoes constitutive phosphorylation by Casein Kinase 2 (CK2) on a series of N-terminal S-D-T repeats and DNA damage-induced phosphorylation by ATM, providing docking sites for NBS1 and the RNF8 ubiquitin ligase, respectively [18–21]. Specifically, the FHA domain of RNF8 recognizes a cluster of highly conserved T-Q-X-F motifs in MDC1, which undergo ATM-mediated phosphorylation upon DNA damage, promoting RNF8 accrual at the damaged chromatin [20,21]. The recruitment of RNF8 and downstream ubiquitin ligase activities to sites of DNA damage then catalyze polyubiquitylation of the core histones H2A and H2AX to trigger a second, ubiquitin-dependent round of protein recruitment to the DSB-flanking chromatin [9,20–23]. The factors arriving with such delayed kinetics include 53BP1, PTIP, and BRCA1-containing protein complexes.

Since the initial discovery of RNF8 and the ubiquitin-dependent DSB signaling response, a large body of work from many labs has revealed a remarkable regulatory complexity of the mechanisms governing histone ubiquitylation at sites of DNA damage. This is based on the identification of a growing number of ubiquitin ligases and associated factors that either participate directly in these reactions or depend on them for their own recruitment [9,15]. Moreover, it has been demonstrated recently that SUMOylation of so far elusive DSB-signaling factors are also required for efficient histone polyubiquitylation at sites of DNA damage to occur [16]. Although the continued efforts at dissecting the molecular basis of this response are likely to turn up yet novel features of its regulatory control, a picture of a dynamic and multi-faceted response regulated at numerous levels is emerging (Fig. 1).

Upon DNA damage, RNF8 engages in an interaction with HERC2, a giant HECT domain E3 ubiquitin ligase of almost 5000 amino acids [24]. Rather than directly ubiquitylating histones at sites of DNA damage, HERC2 appears to assist RNF8 by modulating its preferred choice of cognate E2 ubiquitin conjugating enzyme. Thus, whereas RNF8 has been shown to possess an inherent ability to associate with several E2 enzymes (a property commonly observed for E3 ubiquitin ligases), the association with HERC2 causes RNF8 to selectively interact with Ubc13 to promote the formation of non-proteolytic, K63-linked polyubiquitin chains on histones at sites of DNA damage [24]. In the absence of HERC2, this preference shifts towards other E2s possibly required for other functions of RNF8 in the cell [24]. Besides this established function, it is conceivable that HERC2 may assist RNF8 in additional, as yet unanticipated ways. In particular, it has not yet been addressed whether HERC2 employs its own ubiquitin ligase activity in the DSB-associated chromatin response [24]. However, it is clear from other recent studies of HERC2 that it can function as an active E3 ubiquitin ligase towards factors relevant for DNA repair, including BRCA1 and XPA [25,26]. In addition to the HECT ubiquitin ligase domain, HERC2 also contains 3 clusters of RCC1 repeats, protein domains that in the RCC1 protein (Regulator of Chromosome Condensation 1) function to bind directly to core histones [27]. If the RCC1

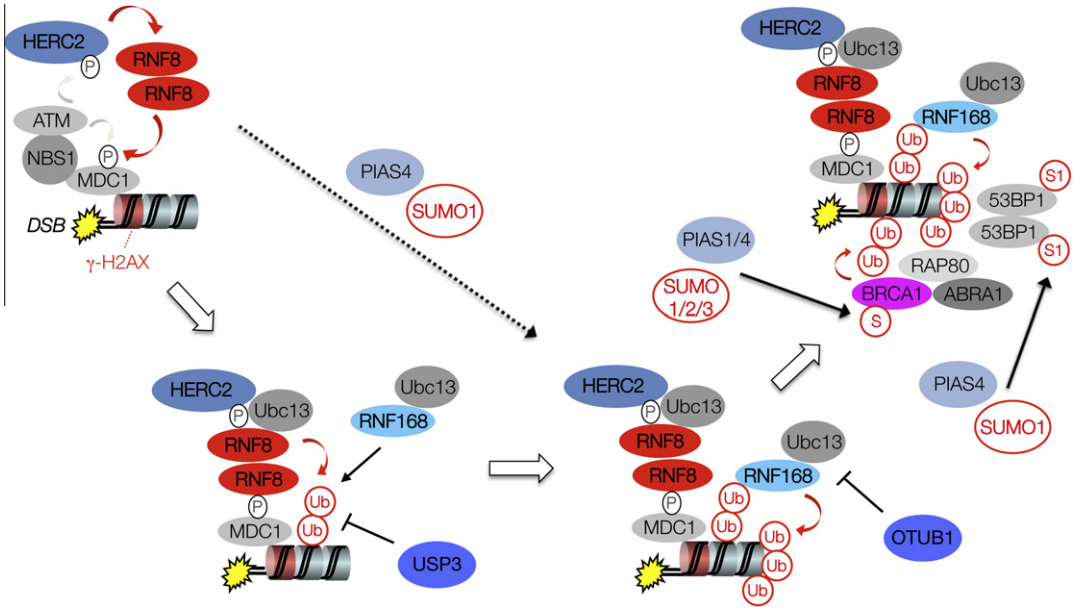

Fig. 1. Ubiquitin- and SUMO-dependent regulation of protein assembly at DSB sites. In response to DSBs, ATM-mediated phosphorylation of γ-H2AX-bound MDC1 and of HERC2 generates binding sites for the RNF8 ubiquitin ligase to promote the formation of a ternary MDC1-RNF8-HERC2 complex at sites of DNA damage. HERC2 facilitates the interaction between RNF8 and Ubc13 to promote initial K63-linked polyubiquitylation of H2A-type histones. This in turn creates binding sites for the MIU domains of RNF168, allowing its recruitment to the damaged chromatin. RNF168 amplifies histone polyubiquitylation of the DSB-flanking chromatin to levels sufficient of allowing accumulation of genome caretaker proteins such as 53BP1 and the BRCA1 A complex at the DSB sites. BRCA1 may promote the ubiquitylation of additional factors present at the DSB-associated chromatin. In parallel with ubiquitylation, the SUMO E3 ligases PIAS1 and PIAS4 promote the SUMOylation of BRCA1 and 53BP1. PIAS4-mediated SUMOylation is also required to allow RNF168 retention at and ubiquitylation of the damaged chromatin by an as yet unknown mechanism. Several DUBs, including USP3 and OTUB1, function as negative regulators of the ubiquitin-dependent chromatin response. P, phosphate; Ub, ubiquitin; S, SUMO; S1, SUMO1.

repeats in HERC2 serve a similar purpose, they could be important for bringing RNF8 into close proximity with its nucleosome and chromatin target(s) as well as its E2 partner Ubc13. The MDC1-RNF8-HERC2-Ubc13 complex constitutes a ubiquitin ligase complex that is assembled on chromatin surrounding DSBs to prime polyubiquitylation of H2A and H2AX [24]. It is largely assembled by ATM-mediated phosphorylations that are recognized by the FHA domain in RNF8. Thus, this FHA domain is able to recognize phosporylated MDC1 on the one hand, as well as a unique ATM phosphorylation site in the extreme C-terminus of HERC2. Due to an additional, inherent ability to di- or oligomerize, RNF8 is able to bridge the interaction between MDC1 and HERC2, thus targeting the whole complex to sites of DNA damage [24].

Despite the presence of HERC2 as an activating co-factor, RNF8 remains a relatively inefficient H2A ubiquitin ligase that only poorly modifies histones in a nucleosome context (our unpublished observations), and which, consequently, is in itself insufficient to promote ubiquitin-dependent retention of genome caretaker factors at the DSB sites. Thus, rather than promoting the bulk of DSB-associated histone H2A and H2AX (H2A(X)) polyubiquitylation, the RNF8-HERC2 complex appears to serve a priming role for this process, paving the way for a downstream E3 ligase, RNF168, which unlike RNF8 displays high activity towards H2A-type histone substrates [28,29]. Like RNF8, RNF168 employs Ubc13 as an E2 partner and catalyzes K63-linked polyubiquitylation of histone H2A(X). RNF168 is attracted to sites of DNA damage via a set of MIUs, a specialized type of inverted ubiquitin interacting motif, which is also found in the Rabex5 protein, and which can bind to both K48- and K63-linked ubiquitin chains [28–31]. By means of its E3 ligase activity, RNF168 amplifies ubiquitylation of H2A-type histones to levels sufficient of promoting the ubiquitylation-dependent recruitment of downstream factors such as 53BP1 and BRCA1 [28,29]. Through its ability to both promote and associate with H2A ubiquitylation, RNF168 may effectively self-reinforce and expand histone polyubiquitylation at the DSB-flanking chromatin. Such two-step model involving the sequential actions of RNF8 and RNF168 is consistent with the finding that RNF168 arrives slightly, but significantly, later than RNF8 at sites of DNA damage in a manner fully dependent on functional RNF8 [28]. Thus, RNF8 plays a crucial role in the pathway by marking sites of DNA damage for RNF168 accrual and setting the stage for this highly active E3 ligase. Of note, RNF168 was recently shown to harbor a third ubiquitin binding domain, termed a UMI domain, which appears to cooperate with the MIUs to efficiently bind ubiquitylated histones at sites of DNA damage [32]. Importantly, reflecting a critical clinical importance of the RNF8/RNF168 signaling pathway, biallelic heterozygous nonsense mutations in the *RNF168* gene was shown to be the underlying genetic defect in a patient with the RIDDLE syndrome, characterized by marked radiosensitivity as well as immunological and neurological defects [29,33]. Recently, a second RIDDLE-like patient with homozygous *RNF168* nonsense mutations resulting in even more truncated RNF168 proteins, and more severe clinical manifestations, was described [34]. It seems likely that mutations in other factors governing DSB-induced chromatin ubiquitylation may underlie other cases of genomic instability disorders for which the molecular basis is unknown, and it will be important to identify such cases in the future.

Whereas the available current evidence suggests that RNF8 precedes RNF168 in the H2A(X) histone polyubiquitylation cascade, a key question is whether RNF168 functions merely to boost such ubiquitylation initiated by RNF8, or whether RNF8 and RNF168 also ubiquitylate additional, and perhaps distinct subsets of proteins involved in facilitating DSB repair. Moreover, it is not clear to which extent E3 ligases other than RNF8 and RNF168 may contribute to DSB-induced ubiquitylation of H2A-type histones. For instance, the ubiquitin ligase BMI1, which forms part of the polycomb repressive complex 1 that promotes H2A monoubiquitylation and transcriptional silencing, was recently found to undergo recruitment to DSB repair foci in an RNF8-dependent manner, and cells depleted of BMI1 display slight sensitization to IR [35,36]. Unlike RNF8, however, BMI1 does not appear to be absolutely required for recruitment of 53BP1 into IRIF, and so the exact function of BMI1 in promoting DNA damage-induced histone ubiquitylation remains unclear. In general, given the considerable number of E3 ligases that have been found to undergo recruitment to IRIF [9], it seems likely that many factors present in these structures may be targeted by ubiquitylation in response to DSBs. At present, only histone proteins including H2A, H2AX, and H2B have been convincingly shown to be subject to such modification. For a number of the IRIF-associated ubiquitin ligases, including BRCA1, HERC2, and RAD18, the nature of their substrates, if any, at these structures, remain largely unknown. Reagents like the FK2 monoclonal antibody, which recognizes conjugated ubiquitin species, is routinely used to detect the accumulation of ubiquitylated products in DSB repair foci, but the precise nature of the modified protein(s) that such antibodies detect is largely unclear and may well include proteins other than histones. Moreover, conflicting evidence exists with regard to the relative contribution of BRCA1 to the ubiquitin products recognized by the FK2 antibody; whereas marginal effects on loss of FK2 reactivity in IRIF upon BRCA1 depletion have been reported [20], other studies found that BRCA1 provides a major contribution to the signal recognized by this antibody [37–39]. Accordingly, it is not clear if and to which extent BRCA1 contributes to DSB-induced histone ubiquitylation initiated by RNF8/RNF168. Resolving this and related issues will be required to define more clearly the individual contributions and roles of RNF8, RNF168, and BRCA1 in the DSB-responsive ubiquitin ligase cascade. Overall, the identification of novel targets of DSB-induced ubiquitylation by specific E3 ligases associated with the DSB-flanking chromatin will be of major importance for a deeper mechanistic insight into how ubiquitin-dependent signaling mechanisms promote cellular responses to DSBs.

Along with ubiquitin, a range of ubiquitin-like modifier proteins expressed in eukaryotic cells also have important functions in numerous cellular signaling pathways [40]. Among these, and similar to ubiquitin, the SUMO proteins have well-established roles in genome stability and repair responses [41]. Recently, work from several laboratories uncovered a critical role for SUMOylation in promoting DSB-associated histone ubiquitylation. Essentially, the SUMO E3 ligases PIAS1 and PIAS4 were shown to be required for recruitment of BRCA1 and 53BP1 to IRIF, and both SUMO1 and SUMO2/3 accumulate in the DSB-associated chromatin areas [38,39]. Likewise, PIAS1 and PIAS4 are themselves recruited to DSB sites, but the mechanistic basis for their retention at damaged chromatin has not been delineated. Both 53BP1 and BRCA1 were found to be direct targets of SUMOylation, and in the case of BRCA1 such modification appears to stimulate the intrinsic E3 ubiquitin ligase activity of the BRCA1-BARD1 heterodimer [38,39]. However, this effect has so far been observed only in vitro using recombinant fragments containing the RING domains of BRCA1 and BARD1, and hence the in vivo significance and possible underlying mechanism for this observation remains to be determined. Interestingly, evidence from these studies also suggested an important and likely more direct function of SUMO in facilitating DSB-induced histone ubiquitylation upstream of 53BP1 and BRCA1 recruitment. To this end, it was shown that accumulation of ubiquitin conjugates at sites of DNA damage requires the activity of the SUMO E3 ligase PIAS4 [38]. Although the mechanisms that govern the relationship between SUMOylation and histone ubiquitylation are entirely unclear at present, it is tempting to speculate that SUMOylation impacts directly on the activity of one or more of the RNF8,

RNF168, and HERC2 ubiquitin ligases. Accordingly, ablation of PIAS4-mediated SUMOylation suppressed RNF168 accumulation at sites of DNA damage, but still allowed for RNF8 accrual [38]. Such discrepancy is typical for interventions (such as HERC2 depletion) that negatively impact on the ubiquitylation reaction without affecting the primary sensing of lesions and ATM-mediated proximal signaling in the DSB-induced chromatin response. Indeed, we find that both RNF168 and HERC2 are directly modified with SUMO in response to DSB formation (our unpublished observations), and hence it is possible that the activities of these E3 ligases may be regulated by SUMO in a manner similar to that of BRCA1. In an alternative scenario, PIAS4 could promote the formation of SUMO conjugates that target RNF8 and/or RNF168 to their substrates. Such a mode of action is known from the SUMO-targeted ubiquitin ligase RNF4, which harbors four closely spaced SUMO-interacting motifs (SIMs) that enable its specific interaction with and subsequent degradation of SUMOylated substrates [42,43]. In a third but equally plausible scenario, direct SUMOylation of a ubiquitin ligase component may induce conformational changes in the protein that alters its ability to recognize its target. Such a mechanism has been shown to govern lesion recognition by the thymine DNA glycosylase enzyme in the Base Excision Repair pathway [44,45]. Regardless of which, if any, of these hypothetical scenarios will ultimately turn out to be correct, a key question for the field will be to uncover the precise mechanism(s) by which SUMOylation triggers RNF8/RNF168-mediated ubiquitylation of the DSB-associated chromatin.

At present, our knowledge of how the DSB-associated ubiquitylation response is activated greatly exceeds our understanding of how its magnitude and duration is controlled. Given the obvious requirement for a tight regulation of the response and the large number of factors that are needed to produce polyubiquitylated histones, it is conceivable that the mechanisms governing this latter aspect of the response are equally elaborate. Thus, a range of counteracting activities in the form of ubiquitin- and/or SUMO-specific proteases are likely to function in reversing or inactivating the process of DSB-induced chromatin ubiquitylation. Mammalian genomes encode an estimated 80–90 deubiquitylating enzymes (DUBs) predicted to be catalytically active [46], and at least two such DUBs have been clearly implicated in negative regulation of the ubiquitin-dependent DSB response. First, USP3 was shown to counteract ubiquitin-dependent IRIF assembly by directly catalyzing H2A deubiquitylation [28,47]. More recently, the deubiquitylating enzyme OTUB1 was also shown to function as a thresholding factor for this response. Surprisingly, however, OTUB1 does not oppose DSB-induced chromatin ubiquitylation by virtue of its catalytic activity towards ubiquitin chains, but rather by suppressing the activity of RNF168 through interaction with and inhibition of Ubc13 [48]. Whether any of the six SUMO proteases (SENPs) expressed in mammalian cells [49] play similar roles in opposing DSB-induced SUMOylation processes to harness the magnitude and duration of the DSB-induced signaling response remains to be addressed.

Histone H2A is potentially the most abundantly ubiquitylated protein in the cell. It is estimated that roughly 10%-15% of chromatin-associated H2A is monoubiquitylated at any given time, and most prominently, it is associated with transcriptional repression at Polycomb loci [50]. The available evidence suggests that RNF8 and RNF168 generate K63-linked, non-proteolytic ubiquitin chains on histones [21,28,29], which differ markedly from the dominant monoubiquitylated form of H2A, and thus uniquely demarcate the sites of DNA damage for recruitment of genome caretaker proteins. Consistent with this idea, Ubc13, the only E2 enzyme known to exclusively catalyze the formation of K63-linked ubiquitin chains, is required for RNF8 and RNF168 function in the DSB response [21,29,51]. In the first wave of protein recruitment to DSB-modified chromatin, ATM activity provides binding sites for various phospho-dependent interactions, most notably that of MDC1 with γ–H2AX [17]. Similarly, polyubiquitylated histones seem to provide a direct interaction platform for at least a subset of factors in the second wave of protein recruitment to the damaged areas, including 53BP1 and BRCA1. How the latter protein feeds on ubiquitylation of the DSB-flanking chromatin is relatively well understood. A subset of BRCA1 molecules is loosely attached to a complex containing the proteins RAP80, Abraxas, BRCC36, BRE and NBA1, comprising the so-called BRCA1 A complex [52]. Of these, Abraxas constitutes the direct interactor of the tandem BRCT domains of BRCA1 by virtue of a phosphorylated S-P-X-F motif [53,54]. RAP80, on the other hand, contains tandem Ubiquitin Interacting Motifs (UIMs), which targets the BRCA1 A complex to sites of DNA damage via a direct interaction with polyubiquitylated H2A [55]. Of note, the linker region between the two adjacent UIMs in RAP80 was shown to position these in an angle that strongly selects for binding to K63-linked polyubiquitin [56,57], thus providing specific affinity of this complex to sites of DNA damage, and not other nuclear sites enriched in ubiquitylated H2A.

As for other factors whose chromatin accrual at DSB sites is dependent on histone polyubiquitylation, such as 53BP1 and PTIP, the recruitment mechanisms and their dependency on ubiquitin are comparatively much less clear. In the case of 53BP1, a chromatin-binding Tudor domain is essential for its DSB association [58]. This domain binds to methylated histones, and a number of studies have suggested that it interacts preferentially with mono-and di-methylated lysine-20 on histone H4 [59–61]. However, as the overall levels of these epigenetic marks do not seem to change significantly after genotoxic insults [58,62], it is not understood how this well-established marker of DSBs recognizes sites of DNA damage. At least two scenarios could be envisaged. In one, 53BP1 contains, or interacts with a protein that confers, ubiquitin-binding capability, similar to that of RAP80. In an alternative scenario, the polyubiquitylation associated with sites of DNA damage brings about a restructuring of chromatin that exposes an increased number of binding sites for the 53BP1 Tudor domain. Recently, a local increase of H4-K20 methylation was detected on chromatin surrounding DSBs, using chromatin immunoprecipitation (ChIP) [63]. Such increase, shown to be mediated by the MMSET histone methyl transferase [63], could potentially account for the increased affinity of 53BP1 for damaged regions of the nucleus. However, this mechanism still falls short of explaining the requirement of histone ubiquitylation for 53BP1 retention at sites of DNA damage.

Histone ubiquitylation has mainly been studied in the context of transcriptional regulation. Consistently, recent findings suggest that RNF8-mediated histone ubiquitylation at sites of DNA damage may also contribute to locally repress transcription [64]. Whether the nature of the ubiquitin species required for such repression are topologically similar or identical to those that promote the accumulation of BRCA1 and 53BP1 at DSB-flanking chromatin is not clear at present, but it is becoming increasingly apparent that nuclear sites of DNA damage are complex hubs for ubiquitylation processes. Thus, in two recent studies, the ubiquitin ligases RNF20 and RNF40 were shown to undergo recruitment to sites of DNA damage, where the RNF20-RNF40 heterodimeric complex subsequently promotes monoubiquitylation of histone H2B [65,66]. This process defines a novel and distinct branch of the ubiquitin-dependent DSB response, as the RNF8/RNF168 pathway appears to mainly target H2A-type histones. Moreover, in contrast to RNF8/RNF168-mediated H2A polyubiquitylation, H2B monoubiquitylation is not uniquely present at sites of DNA damage. In fact, this type of modification is highly abundant in the nucleus of unperturbed cells, and is normally associated with stimulation of transcript elongation [50]. Indeed, the DSB-induced increase in H2B monoubiquitylation could only be detected once the bulk of constitutive H2B ubiquitylation had been artificially depleted by

the administration of transcription inhibitors [65]. Rather than stimulating transcription, DSB-associated H2B ubiquitylation is proposed to stimulate DNA repair through its positive effect on chromatin decompaction, which may at least in part involve the SNF2 h/SMARCA5 chromatin remodeling factor [65,66]; the precise mechanistic nature of this process, however, remains to be defined. Moreover, while the degree of crosstalk and functional interplay between the RNF8/RNF168- and RNF20-RNF40-mediated DSB responses is not clear at present, these pathways appear to operate in a largely independent fashion from each other. Hence, the RNF8/RNF168 pathway is dispensable for RNF20-RNF40-mediated H2B monoubiquitylation in response to DSBs [65]. Likewise, RNF20-RNF40 is not required for the recruitment of genome caretaker proteins such as 53BP1 and BRCA1 to DSB sites [65]. Finally, unlike the DSB-associated H2A(X) polyubiquitylation, which is qualitatively distinct from the much more abundant H2A(X) monoubiquitylation mark found throughout the nucleus, there is currently no evidence to indicate that H2B undergoes polyubiquitylation in response to DSBs [65,66]. This may in turn suggest that the DSB-induced H2B monoubiquitylation does not serve as a chromatin receptor for one or more DSB repair factors, but instead has a more direct, stimulatory effect on local chromatin decompaction to facilitate access of DNA repair factors to the damaged DNA.

Collectively, work from many laboratories has established that the DSB-associated chromatin areas constitute a complex landscape of ubiquitin- and SUMO-modified proteins. In particular, as discussed above, whereas the number of known ubiquitylated and SUMOylated substrates at DSB sites remain limited, many ubiquitin and SUMO E3 ligases have been found to undergo recruitment to IRIF, and additional ones are likely to follow. Hence, it will be important and exciting to identify and profile additional ubiquitin- and SUMO-dependent signaling processes operating in the context of inflicted DSBs, and to dissect how these events contribute to the reestablishment of genome integrity following such lesions.

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